NOVEL IMMOBILIZED-BIOCATALYST BIOREACTORS FOR PRODUCTION OF FUELS AND CHEMICALS

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ABSTRACT

There are known biocatalytic pathways to produce many common fuels and petrochemicals. These biocatalysts can be either enzymes or living microorganisms. The challenge is to produce these fuels and chemicals efficiently and economically. Critical parameters include feedstock costs, yield, rate and downstream processing. Here we will examine several immobilized biocatalyst reactor designs that will increase overall rates. We will also discuss extractive bioreactors designed to decrease downstream separation cost by directly removing the dilute inhibitory products. Illustrative examples tested at ORNL will include ethanol production, extractive fermentation to butanol, and nonaqueous enzymatic bioconversions.

Keywords: Ethanol, bioreactors, non-aqueous biocatalysis.

INTRODUCTION

Bioconversion processes utilize a biocatalyst (microorganism, enzyme, or other active fraction) to enhance the conversion of a feed material or substrate to a useful product in a controlled environment. It is particularly desirable for such a system to have high volumetric productivity with maximum concentration and yield of the product. Continuous operation with good process control is also desirable. At least two subcomponents need to be considered: the production of the bioreagent and the bioconversion reactor itself.

Most bioconversion processes utilize a soluble substrate in an aqueous solution and produce a product that is also soluble in the aqueous phase. However, the substrate can be a solid, such as cellulose or starch or even gases, such as syngas or methane. Similarly, the products can be solids, liquids, or gases. The reaction medium can be an aqueous solution, a moist gas, or even an organic liquid in contact with the biocatalytic component. An efficient biocatalyst system must be available in a bioreactor configuration that optimizes interphase contact, mass transport, and conversion kinetics.

Characteristics of an advanced bioreactor should include, if possible, a high concentration of the biocatalyst, continuous operation, and excellent contact between the reacting components. Many bioreactor configurations have been proposed and are listed in Table 1. Ethanol production has been carried out in many of the bioreactors and the volumetric productivity for each is also shown. The literature values (1,2) indicate that cell retention can provide substantial increases in productivity. The conventional bioreactor system today is a large stirred tank operating in the batch mode usually with microorganisms or enzymes in aqueous suspension as the biocatalyst. Downtime between batches can decrease the overall productivity of these processes. However most of the productivity gains are from the high levels of biocatalyst possible in retained systems. Retaining and "reusing" the biocatalyst will also decrease the costs due to the enzyme or microbe itself and may be essential for an economic process.

There are a variety of methods to retain the biocatalyst within the system. Here we will divide the discussion of novel systems between aqueous based immobilized-cell systems and nontraditional nonaqueous biocatalytic system (3). Serious consideration is now being given to the use of biocatalytic systems in or in contact with nonaqueous media. These primarily include organic solvents and supercritical liquids (4). However, reactor concepts for these systems are only now being developed. A discussion of aqueous based cell retention systems tested at ORNL will be followed by nonaqueous systems using solvents or vapors.

Table 1. Ethanol production in various bioreactors.
[Productivities were at >95% conversion (1,2)]

Reactor	Productivity ($g\ L^{-1}\ h^{-1}$)
Batch	1.8-2.5
CSTR	6-8
Batch w/cell recycle	6-7
CSTRs in series	10
CSTR w/cell recycle	10-15
Hollow fiber	15-30
Immobilized-cell CSTR	10-20
Immobilized-cell packed column	10-50
Immobilized-cell fluidized-bed	20-100

AQUEOUS-PHASE RETAINED BIOCATALYSTS

One method to retain microbes in a continuous process is cell recycle using centrifugation or membrane. However, an alternative to a conventional CSTR with cell recycle is the use of retained biocatalysts by immobilization onto integral parts of the reactor or by immobilization into or onto solid particles that will be kept in the bioreactor even at high flow rates. Two primary approaches can be used: 1) adsorption or attachment of the biocatalyst to external or internal surfaces of the solid phase; or 2) encapsulation of the biocatalyst within the particulate matrix or media (5). This can result in a very high concentration of the biocatalyst that does not wash out of the bioreactor. Here the biocatalyst production step becomes a separate process for the production of large amounts of biomass or enzymes. Although the retained-cell concept can be used in stirred tanks, it is even more effective when used in columnar bioreactors.

Many commodity chemicals can be produced by fermentation. Research at ORNL has emphasized those systems that operate continuously with high volumetric productivity, which are most promising. Columnar bioreactors with retained biocatalysts have been particularly attractive, and three of these reactors are now described and compared with other systems.

ETHANOL PRODUCTION IN A FLUIDIZED-BED BIOREACTOR

In prior efforts at ORNL, immobilized *Zymomonas mobilis* was used in FBRs for high productivity and conversion production of ethanol (6). The bacteria were immobilized within small uniform gel beads (~1-mm diam.) at cell loadings of up to 50 g dry wt/L. Conversion and productivity were measured under a variety of conditions, feedstocks, flow rates, and column sizes (up to 8 ft tall). Volumetric productivities of 50 to 100 g ethanol L^{-1} h^{-1} have been achieved with residual glucose concentrations of <0.1%. The biocatalyst beads have been shown to remain active for over 2 months. This technology has several advantages over conventional batch technology. Immobilization increases volumetric productivity by increasing cell density. The use of beads of near 1-mm diam. minimizes the effect of mass transfer resistances. Fluidization allows for good interphase mass transfer and the release of large volumes of coproduct CO_2. The columnar operation allows multistage operation and localizes the high inhibitory product concentrations to the top of the reactor. This would allow a much smaller reactor with smaller capital costs to be used for the same alcohol output. Another advantage of this FBR was the operation without asepsis. A major advantage was the improved ethanol yield per gram dextrose of 0.49 g/g or >97% of the theoretical stoichiometric limit of *Z. mobilis* compared to a yield of 0.45 to 0.47 g/g for yeast. Under current economic conditions, the raw materials (i.e., dextrose from corn or other sources) are the largest single part of the cost; therefore, even a small but consistent increase in the yield can result in appreciable savings over the expected FBR operating lifetime of months.

Recently this concept was extended to a combined process with two concurrent reactions. The production of ethanol from industrial dry-milled cornstarch was studied in a laboratory-scale fluidized-bed bioreactor using immobilized biocatalysts. (7) Saccharification and fermentation were carried out either simultaneously or separately (see Figure 1). Simultaneous saccharification and fermentation (SSF) experiments were performed using small, uniform κ-carrageenan beads (1.5 to 2.5 mm in diameter) of co-immobilized glucoamylase and *Z.mobilis*. Dextrin feeds obtained by the hydrolysis of 15% dry-milled cornstarch were pumped through the bioreactor at residence times of 1.5 to 4 h. Single-pass conversion of dextrins ranged from 54 to 89%, and ethanol concentrations of 23 to 36 g/L were obtained at volumetric productivities of 9 to 15 g L^{-1} h^{-1}. Very low levels of glucose were observed in the reactor, indicating that saccharification was the rate-limiting step. In separate hydrolysis and fermentation (SHF) experiments, dextrin feed solutions of 150 to 160 g/L were first pumped through an immobilized-glucoamylase packed column. At 55°C and a residence time of 1 h, greater than 95% conversion was obtained, giving product streams of 162 to 172 g glucose/L. These streams were then pumped through the fluidized-bed bioreactor containing immobilized *Z. mobilis*. At a residence time of 2 h, 94% conversion and ethanol concentration of 70 g/L were achieved, resulting in an overall process productivity of 23 g L^{-1} h^{-1}. At residence times of 1.5 and 1 h, conversions of 75 and 76%, ethanol concentrations of 49 and 47 g/L, and overall process productivities of 19 and 25 g L^{-1} h^{-1}, respectively, were achieved.

EXTRACTIVE FERMENTATIONS WITH IMMOBILIZED CELLS

Many commercial organic acids and solvents, such as acetic, citric, lactic, and succinic acids, can be produced by fermentation (8). All are produced in relatively dilute form due to their high level of inhibition of the microorganism. This inhibition is due to both the chemical itself and the lowered pH from acid production. Improvements in rate have been observed using various means of cell retention including cell recycle, membranes, and immobilization.(9, 10) Several processes have been proposed to remove the inhibitory product from the ongoing fermentation.(11) The key advantages suggested for extractive bioconversion are higher feed concentrations leading to less process wastes and reduced product recovery costs compared to those of distillation. Possibilities for in situ product removal include pervaporation, the use of

hollow-fiber reactors, and the use of solid adsorbents as well as the use of an immiscible extractive solvent. Key issues are the extractant toxicity and capacity as well as the actual contacting scheme devised and its operability. Adsorption has been proposed in various forms to remove the acids from the broth. This has included direct addition into the batch STR (with problems of attrition and power); passing a broth recycle stream through a side adsorbent bed; and a direct addition and removal of the adsorbent to a fluidized bed of immobilized biocatalysts.(12)

This biparticle FBR has been tested for simultaneous fermentation and separation of lactic acid. (13) The bioreactor is a fluidized bed of immobilized *Lactobacillus delbreuckii*. Another solid phase of denser sorbent particles (a polyvinyl pyridine resin) was added to this fluidized bed. These sorbent particles fell through the bed, absorbed the product, and were removed. In test fermentations, the addition of the sorbent enhanced the fermentation and moderated the fall of the pH. The biparticle FBR utilizing immobilized microorganisms and adsorbent particles has been shown to enhance the productivity of lactic acid to 7 g L^{-1} h^{-1} – a sixteenfold increase over a control batch fermentation in this nonoptimized system. Regeneration of the sorbent allowed significant concentration of the product.

Most studies of extractive acetone-butanol fermentation have been performed in a batch reactor (14) with free cells. An immobilized-cell FBR with a cocurrent immiscible liquid extractant(15) demonstrated a significant 50 to 90% increase in butanol production rate and yield in a nonoptimized extractive FBR system compared to the nonextractive FBR. The extractant oleyl alcohol removed most of the butanol from the aqueous phase during an active fermentation in a fluidized bed with immobilized *C. acetobutylicum* for the acetone-butanol fermentation. Under continuous, steady-state operation, the butanol yield increased to 0.3 g/g with a productivity of 1.8 g L^{-1} h^{-1} when butanol was removed in this manner.

NONAQUEOUS BIOCATALYSIS

Enzymatic reactions in a nonaqueous phase offer a number of advantages over traditional aqueous based processes, including elimination of undesirable side reactions, more favorable thermodynamic equilibria and simplified product recovery. Most nonaqueous biocatalysis has been performed with placing the enzyme in an organic solvent. The enzyme may be insoluble in the solvent. Many proteins precipitate and are inactivated by solvents and so may require modification to increase their solubility and activity. Hydrophobic groups such as polyethylene glycol can be chemically added to the protein to increase its solubility while retaining catalytic activity (16).

Surprizingly, "dry" enzymes can also retain catalytic activity directly to vapors. In this case the enzyme or biocatalyst is a "solid" catalyst on which the enzyme binds and reacts. Bench scale reactors were operated in continuous recycle and single pass modes using immobilized porcine lipase to catalyze gas-phase esterification of ethyl alcohol with two carboxylic acids (acetic acid and propionic acid). (17) Order of magnitude rate increases (over uncatalyzed reactions) in conversion were achieved. Product concentrations ranged from 0.1 to 0.5 mM in air and were strongly affected by substrate concentration and acid induced enzyme inactivation. We have continued these efforts with tranesterification reactions.

CONCLUSIONS

Immobilized-biocatalysts have been demonstrated to be a valuable class of advanced bioreactors for aqueous fermentations. They provide continuous operation, high biocatalyst concentrations, and good interphase mass transfer; thus resulting in higher productivity and often improved product yields. The improved yields may be due to the cell retention by immobilization, which allows less substrate to go to the production of more biocatalyst and thus more can go to product. This has been shown in four configurations here, two including in situ product removal. Nonaqueous systems may offer certain advantages and efforts to develop immobilized biocatalyst systems are just beginning. Further effort is still needed to scale-up and commercialize these attractive designs.

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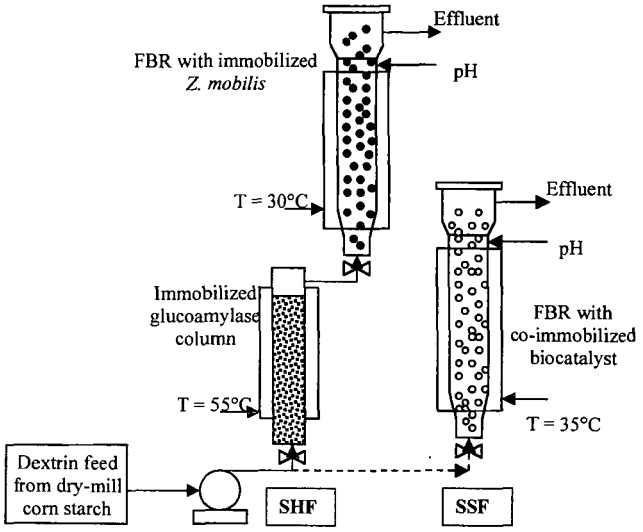

Figure 1. Process schemes for direct fermentation of starch into ethanol via immobilized cells. SHF is separate hydrolysis and fermentation, SSF is simultaneous fermentation and separation.